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Research Article

New records of *Polistes* paper wasps (Hymenoptera: Vespidae) for Georgia with first DNA barcodes from Vashlovani National Park

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Abstract

We hereby present the first country records of two vespid species of the genus *Polistes* Latreille, 1802 (Vespidae) — *Polistes bucharensis* Erichson, 1849 and *P. mongolicus* du Buysson, 1911 — for Georgia (Sakartvelo). We identified specimens morphologically and confirmed the identification with DNA barcode data. Based on this, we provide some of the first DNA barcodes for these species from the region as a basis for future research.

Key words: Biodiversity hotspot, Caucasus, integrative taxonomy, Sakartvelo

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Introduction

Georgia is a country in the heart of the Caucasus and one of the world's biodiversity hotspots. Within the country, Vashlovani National Park (hereafter as Vashlovani NP) is located in the extreme southeastern part of Georgia (Sakartvelo), in the Kakheti region next to the Azerbaijan border, and boasts diverse natural features. The park encompasses several distinct zones, including steppe, forest-steppe, semi-desert, and deciduous forests.

Vashlovani NP areas have a dry climate, with elevations ranging from 95 to 900 meters above sea level. Summers are hot and dry. Winters are cooler, yet temperatures rarely drop below 0 °C. The local climate favors a rich community of Hymenoptera, and new finds are constantly being made (Japoshvili and Haris 2024; Riedel and Japoshvili 2025; Japoshvili and Salakaia 2026).

Hymenoptera are a diverse group of insects, currently even considered the most diverse of all insects (Ulrich 1999). Within the Hymenoptera, the parasitoid wasps are the most species-rich; however, the mostly larger Vespidae are more iconic and are generally also better studied. In Georgia, 104 species of Vespidae are known so far (Kobakhidze 1966; Japoshvili and Ljubomirov 2021). Within the family, the genus *Polistes* Latreille, 1802, is a cosmopolitan genus of umbrella paper wasps, comprising about 200 species worldwide (Turillazzi and West-Eberhard 1996). The species of *Polistes* are largely predatory and

consume large numbers of different insects, which is why they are often considered beneficial. However, some polistine wasps (*Polistes dominula* (Christ, 1791) and *Polistes gallicus* (Linnaeus, 1767)) collect nectar for their energetic demands and for the provision of the brood (Kovac et al., 2019).

In Georgia, the genus has received relatively little attention so far. The first studies and information on its presence in Georgia were provided by Radde (1899), who reported *P. gallicus* (Linnaeus, 1767) from Borjomi. Later, Kobakhidze (1956, 1966) added six more species for Georgia: *P. atrimandibularis* (Zimmermann, 1930), *P. biglumis* (Linnaeus, 1758), *P. foederatus* Kohl, 1898, *P. nimpha* (Christ, 1791), *P. sulcifer* (Zimmermann, 1930), and *P. semenowi* (Morawitz, 1889). Two of these, *P. foederatus* and *P. nimpha*, were also recorded from Vashlovani NP (Kobakhidze 1966). One more species, *P. dominula*, was recorded later by Japoshvili and Ljubomirov (2021). Based on these publications, eight species have currently been documented for the country. Here, based on a larger sampling campaign, we add two additional species to the local fauna, raising the total number of species to ten.

Material and methods

Sample collection and processing

Specimens were collected within the project "Hymenopteran biodiversity of the Vashlovani National Park" by the team from the Invertebrate Research Center. Every 20 days, from the beginning of April to the end of August, we placed four colored traps (plates) of similar diameter in different colors (yellow, white, blue, and pink) at two locations (Fig. 1). Traps were located in two different habitats of Vashlovani NP: Mijniskure (41.111663, 46.649028) and at the central bungalows (41.159442, 46.567060).

Traps were set on the ground and filled with water (100–150 ml) and a drop of unscented detergent to break surface tension. After 24 hours, the material was collected with tweezers and preserved in vials with 96% ethanol for further study. The species were morphologically identified by the second author using the key by Schmid-Egger et al. (2017). Images of specimens were taken with an Optika Microscopes-ITALY CP-20 camera mounted on a Zeiss Stemi 508 stereomicroscope.

Description of sampling sites

Mijniskure

The Alazani River and its tributaries provide essential water sources for the region. This site features a mix of forests, grasslands, and wetlands (Fig. 1A–C). The natural vegetation of Mijniskure is characterized by diverse vegetation types dominated by *Quercus*, *Pistacia*, *Carpinus*, *Populus*, *Punica*, *Rosa*, *Scirpus*, and *Rubus*. The climate varies depending on elevation and proximity to the Caspian Sea. The region experiences moderate precipitation throughout the year, with rainfall being more abundant in spring and autumn. These mountains are geologically diverse, featuring sedimentary rocks, volcanic formations, and

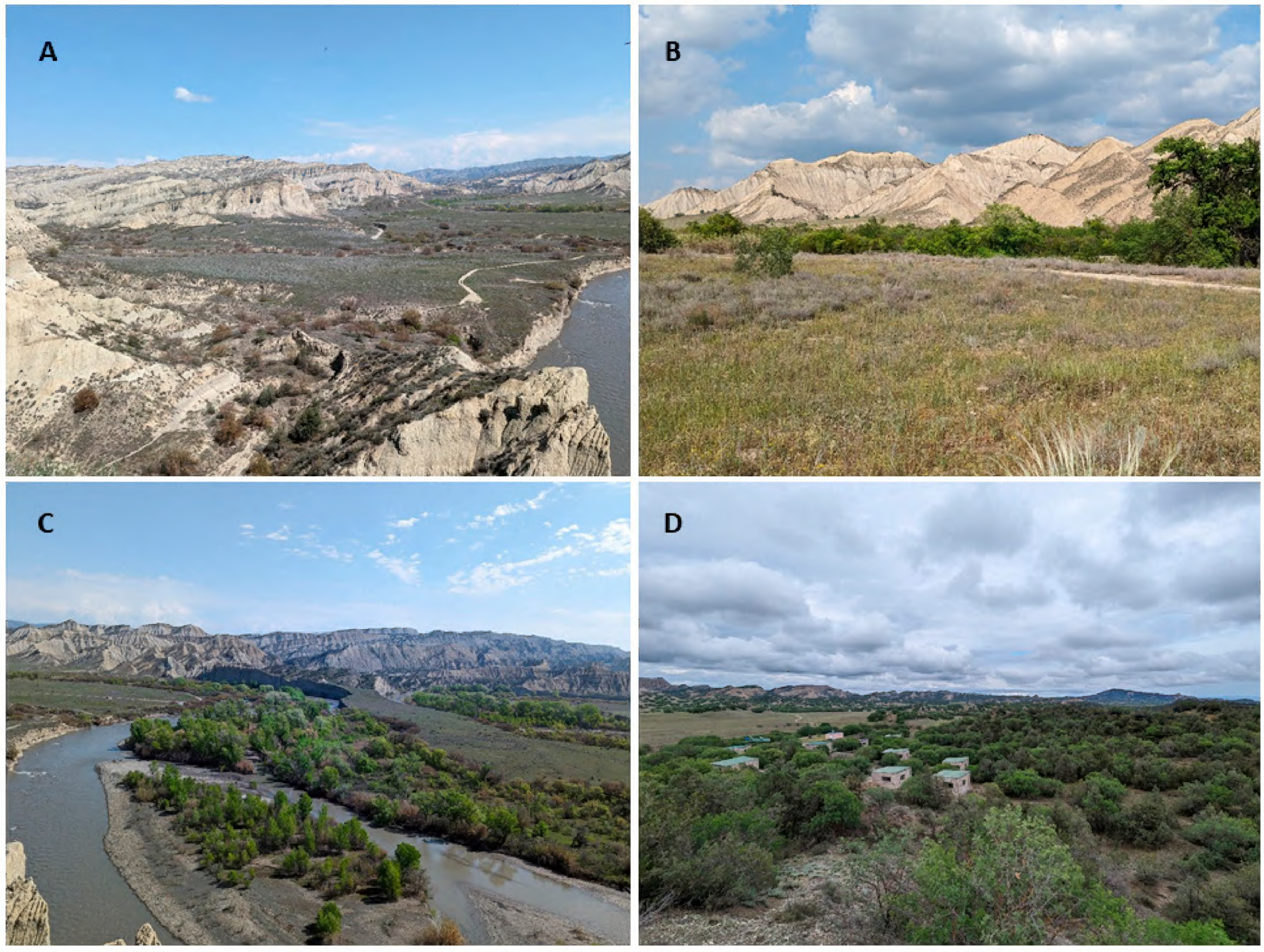

Figure 1. Habitats at sampling sites of Mijniskure (**A,B,C**) and Central Bungalows (**D**).

metamorphic rocks. The Alazani River has shaped the landscape over millennia, carving deep valleys and depositing fertile alluvial soils (APA 2026).

The Central Bungalows

This site contains 11 bungalows built from stone, surrounded by *Pistacia* and *Juniperus* trees (Fig. 1D). Four species of *Juniperus* characteristic of this park are found together here: *J. foetidissima* Willd., *J. rufescens* Link., *J. oblonga* M. Bieb., and *J. polycarpos* K. Koch (Akhalkatsi and Tarkhnishvili 2012).

DNA Processing

Four *Polistes* specimens belonging to three species from Georgia were subjected to DNA barcoding. Genomic DNA was extracted from the middle legs using the Oxgen Soft Tissue DNA Purification Kit (Oxgen, Mtskheta, Georgia) following the manufacturer's protocol. Partial sequences of cytochrome c oxidase subunit I (COI) were amplified by polymerase chain reaction (PCR) using the primer pair LCO1490 and HCO2198 (Folmer et al. 1994). Thermal conditions included denaturing at 95 °C for 1 min, followed by a first cycle set (15 cycles): 94 °C for 30 sec, annealing at 55 °C for 1 min, and extension at 72 °C

for 1:30 min. A second set of cycles was used as follows (25 cycles): 94 °C for 35 sec, 50 °C for 1 min, 72 °C for 1:30 min, and a final extension step at 72 °C for 10 min. PCR products were stored at 4 °C and visualized on 1% agarose gels. PCR products were purified with ExoSAP-IT (Thermo Fisher, Waltham, Massachusetts) according to the manufacturer's protocols. Sequencing of the purified PCR products in both directions was conducted at BGI Tech Solutions (Hong Kong).

We generated further new reference sequences from Georgia (2 × *P.* cf. *gallicus*, *P.* cf. *semenowi*, *P. associus*, *P. bucharensis*), Bulgaria (*P. nimpha*), Armenia (*P. mongolicus*), and Iran (*Polistes* sp.). DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, but with an extended overnight incubation time at 56 °C (Thermomixer, with gentle shaking) for cell lysis. For elution, 100 µL of AE buffer was used. The extracted DNA was stored at −20 °C until use. Primers used for amplification and sequencing were LCO1490-JJ and HCO2198-JJ (Astrin and Stüben 2008). The amplified mitochondrial region is a part of the cytochrome c oxidase subunit I gene (COI) and corresponds to the standard barcode region of the animal kingdom (Hebert et al., 2003).

PCR reactions were carried out in a total volume of 25 µL containing 1.0 µL DNA, 2.0 µL of primers (5 pmol each), and 12.5 µL of 2× Multiplex PCR Plus Master mix (QIAGEN). The PCR protocol consisted of an initial DNA polymerase (HotStar Taq) activation step at 95 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 90 s at 49 °C, and 90 s at 72 °C; the last cycle was followed by a final 10 min extension step at 72 °C. 3 µL of the PCR product were visualized on a 1% agarose gel. The PCR product was purified using 3 M sodium acetate and ethanol precipitation. Sanger sequencing was performed using the BigDye Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems, Thermo Fisher Scientific Inc.) with a total amount of 20 ng of purified PCR product. To obtain unequivocal sequences, both sense and antisense strands were sequenced.

The obtained sequences were trimmed, checked, and aligned using the MUSCLE algorithm implemented in Geneious Prime v. 2022.1.1 (http://www.geneious.com). The remaining DNA extracts were deposited in the scientific collection of the Institute of Entomology, Agricultural University of Georgia, Tbilisi, Georgia. Newly generated DNA barcodes had a length of 648 bp and were compared against the BOLD database to confirm morphological species identification (Ratnasingham and Hebert 2013).

Furthermore, we provided phylogenetic background by performing Bayesian phylogenetic analysis using our 12 newly generated and an additional 21 ingroup sequences of related species taken from BOLD (all newly generated sequences were submitted to NCBI GenBank (www.ncbi.nlm.nih.gov; under the accession numbers: PX502332–PX502343). We included all known species from Georgia for which publicly available sequences exist. A sequence of *Vespa crabro* Linnaeus, 1758, was used as the outgroup (GenBank Accession Number: ACUFI813). Analyses were performed with MrBayes v. 3.2.6 (Ronquist et al. 2012). We used the reversible jump model to provide high flexibility in the analyses and ran the analyses for 1,000,000 generations, sampling every 100 trees to generate a sample of 10,000 trees. Convergence was confirmed by average split frequencies below 0.05. The resulting consensus tree was summarized using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Morphological and genetic identification yielded new records for Georgia: *Polistes bucharensis* Erichson, 1849 (Fig. 2) and *P. mongolicus* du Buysson, 1911 (Fig. 3) were confirmed with genetic results (Fig. 4). We also reconfirmed the presence of *P. foederatus* based on morphological and genetic evidence (Fig. 3). *Polistes nimpha* could only be identified based on morphological characters (Fig. 3), as DNA sequencing was not successful for the specimens from Georgia.

Genetic results

We generated new sequences for the genus *Polistes* from Georgia and constructed a phylogenetic tree, including published sequences from the same species from other locations and related species to confirm identification (Fig. 3). In regard to the outgroup, all *Polistes* formed one cluster with high support. With the exception of one specimen of *P. dominula* (BCHYM12109) grouping with *P. bucharensis* and a specimen of *P. associus* grouping within *P. nimpha*, all species formed monophyletic groups. *Polistes biglumis*, with two specimens of *P.* cf. *gallicus*, as well as *P. gallicus* and *P. mongolicus*, were the basal taxa, but formed a polytomy with another group that included all other taxa. This group is further divided into *P. foederatus*, including our specimen from Georgia. Next, *P. nimpha* splits off, together with one specimen identified as *P. associus*. The last cluster splits into two groups: one of *P. dominula* and *P. bucharensis* (including two sequences from Georgia). However, one specimen identified as *P. dominula* groups at the base of the *P. bucharensis* branch, albeit with low support. The sister group to these two species consists of *P. atrimandibularis*, which is again basal to *P. sulcifer* and *P. semenowi*, which formed a cluster.

The two specimens breaking up species monophylies may be the result of misidentifications, as the respective close relatives are morphologically very similar. In all other cases, morphological and genetic assignments are in agreement.

Faunistic results

Polistes bucharensis Erichson, 1849, new country record

Fig. 2 A–F

Material examined. GEORGIA • Dedoplistskaro, Vashlovani NP, Mijniskure; 41.111663, 46.649028; 95 m a.s.l.; HYM1000038; 24–25.07.2023; color traps, Leg. G. Japoshvili (Fig.1A, D); • Same data, Central bungalows; 41.159442, 46.567060; 275 m a.s.l.; HYM1000051; 8–9.06.2023, color traps, leg. G. Japoshvili (Fig. 1B, E); Same data, HYM1000111; 24–25.07.2023, color traps, leg. G. Japoshvili (Fig. 1C, F).

Genetics. We obtained two DNA barcodes (Genbank Accession Numbers: PX502340, PX502341), which matched data base entries and clustered with specimens identified as the respective species in the phylogenetic tree (Fig. 4).

Distribution. W and C Asia, Cyprus, Egypt and Crete (Schmid-Egger et al. 2017).

***Polistes foederatus* Kohl, 1898**
Fig. 3A, D

Material examined. GEORGIA • Dedoplistskaro, Vashlovani NP, Central bungalows; 41.159442, 46.567060; 275 m a.s.l.; HYM1000052; 8–9.06.2023, color traps, leg. G. Japoshvili.

Barcoding. We obtained one DNA barcode (Genbank Accession Number: PX502342), which matched data base entries and clustered with specimens identified as the respective species in the phylogenetic tree (fig. 4).

Distribution. N Italy to SE Europe, Turkey and Central Asia (Schmid-Egger et al. 2017).

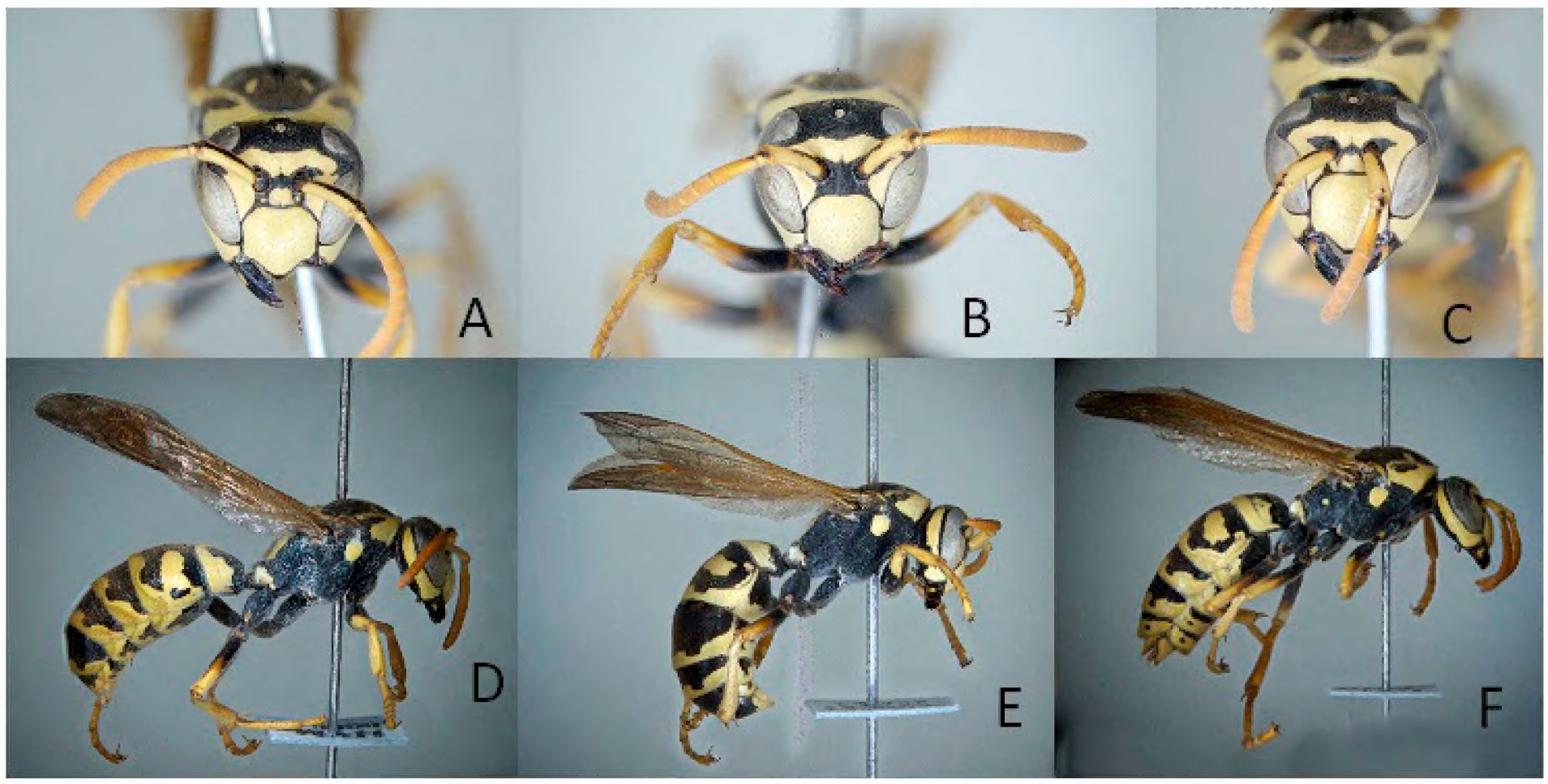

Figure 2. Frontal (**A–C**) and lateral view (**D–F**) of *Polistes bucharensis* representing different morphotypes.

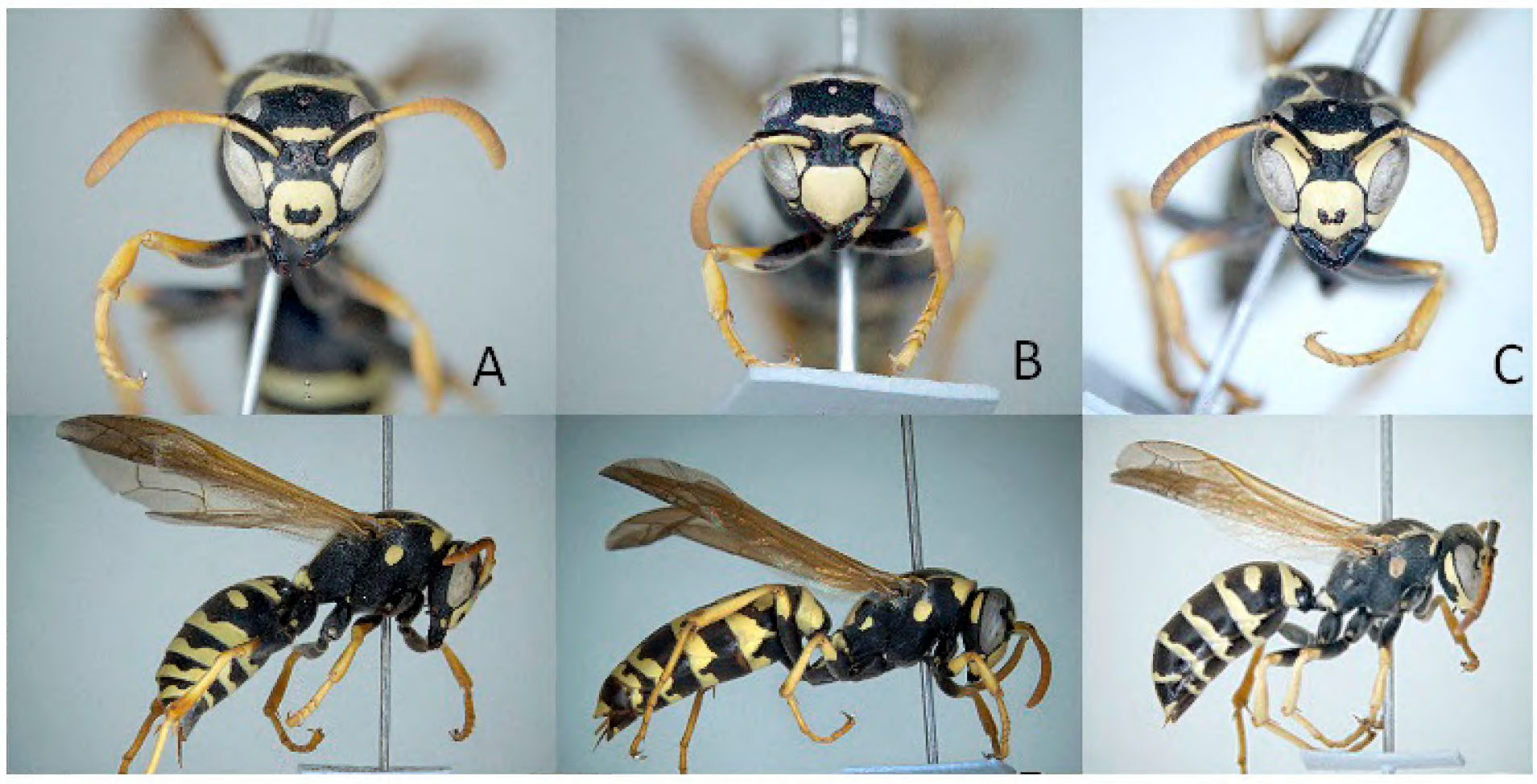

Figure 3. Frontal and lateral views of *Polistes foederatus* (**A, D**); *Polistes mongolicus* (**B, E**); and *Polistes nimpha* (**C, F**).

***Polistes mongolicus* Du Buysson, 1911, new country record**
Fig. 3B, E

Material examined. GEORGIA • Dedoplistskaro, Vashlovani NP, Mijniskure; 41.111663, 46.649028; 95 m a.s.l.; HYM1000035; 5–6.04.2023; color traps, leg. G. Japoshvili.

Barcoding. We obtained one DNA barcode (GenBank Accession Number: PX502343), which matched data base entries and clustered with specimens identified as the respective species in the phylogenetic tree (Fig. 4).

Distribution. Croatia and SE Europe, Turkey, Cyprus to Central Asia and Egypt (Schmid-Egger et al. 2017).

***Polistes nimpha* (Christ, 1791)**
Fig. 3C, F

Material examined. GEORGIA • Dedoplistskaro, Vashlovani NP, Mijniskure; 41.111663, 46.649028; 95 m a.s.l.; HYM1000086; 9–10.06.2023; color traps, leg. G. Japoshvili.

Distribution. Europe and Palearctic Asia (Schmid-Egger et al. 2017).

Discussion

Eight species of the genus *Polistes* had previously been recorded from Georgia. With the addition of two new country records in the present study, the known *Polistes* fauna of Georgia increases to ten species. We generated the first DNA barcode sequences for this genus from the Caucasus region, using these data to confirm species identities against publicly available records (Fig. 4). *Polistes bucharensis* was confirmed with >99.5% sequence identity

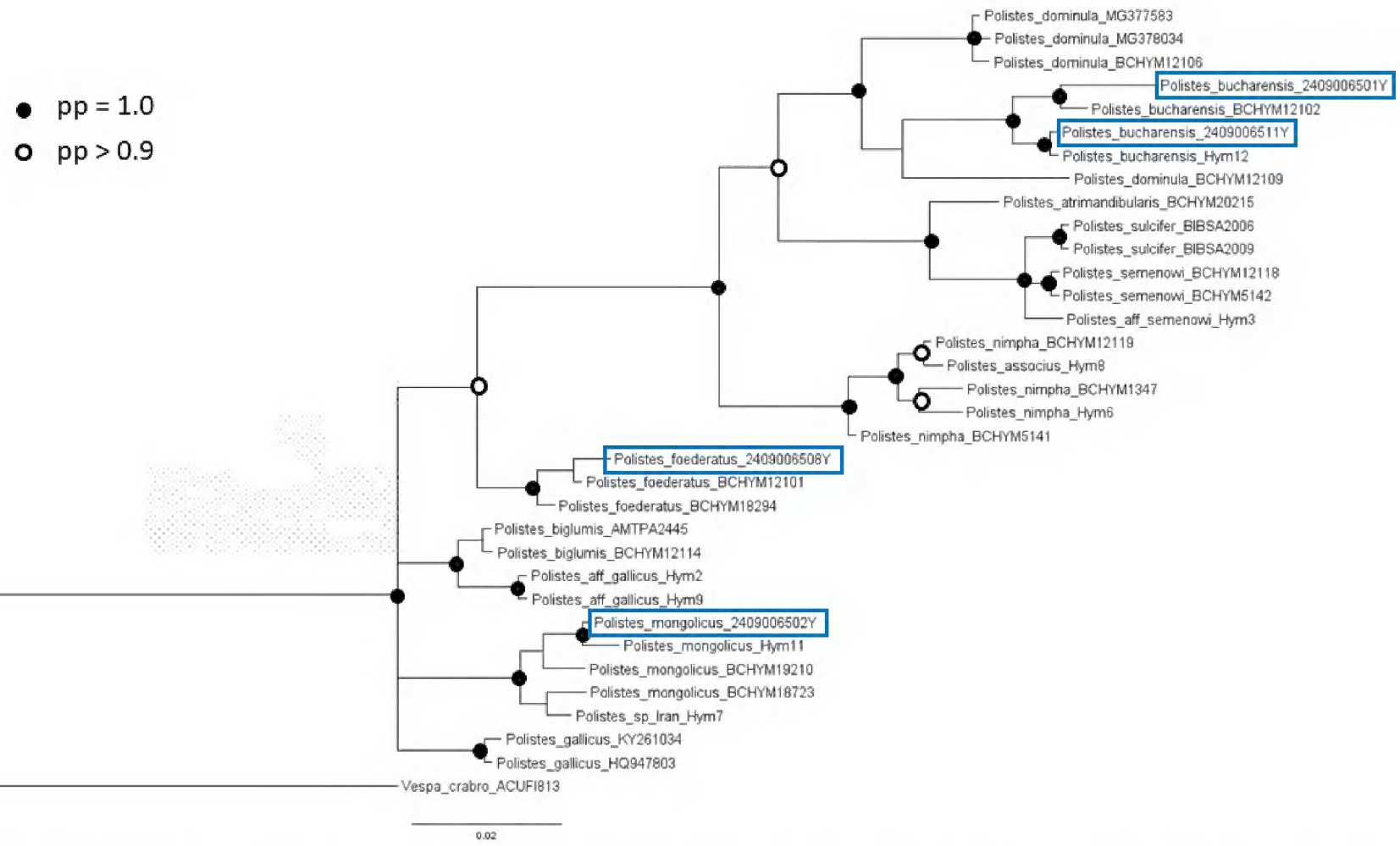

Figure 4. Phylogenetic tree based on Bayesian Inference. Specimens marked with blue frames represent the newly generated sequences.

(specimens 2409006501Y and 2409006511Y), while *P. foederatus* (specimen 2409006508Y) and *P. mongolicus* (specimen 2409006502Y) both showed 100% identity. These close matches are further supported by our Bayesian phylogenetic analysis (Fig. 4), where Georgian specimens formed well-supported monophyletic clades with conspecific sequences. While COI barcoding effectively resolved species boundaries for most taxa, two exceptions were noted, likely reflecting misidentifications of morphologically similar congeners in public databases.Overall, our results represent a significant step toward an integrative assessment of *Polistes* diversity in the Caucasus. As a recognized biodiversity hotspot, this region continues to yield new distributional records and will likely provide further faunistic surprises.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

GJ and TL did fieldwork; GJ and DK did laboratory work; MH did data analyses; GJ, TL, MH wrote manuscript; all authors approved manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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